UNIVERSITY OF CALIFORNIA SAN DIEGO

Evidence of Photoenhancement by Green Fluorescent Proteins in Low Light Conditions: A Potential Factor of Habitat Distribution for the Sea Anemone *Anthopleura sola*

A Thesis submitted in partial satisfaction of the requirements for the degree Master of Science

in

Marine Biology

by

Kelly Govenar

Committee in charge:

Professor Eric Allen, Chair
Professor Nicholas Holland
Professor Kaustuv Roy

2021

The Thesis of Kelly Govenar is approved, and it is acceptable in quality and form for publication on microfilm and electronically.

University of California San Diego

2021

DEDICATION

I would like to dedicate this thesis to all of my friends and family who have supported me along my journey. My dreams of becoming a marine biologist would not have come true without your love and encouragement. I am eternally grateful.

EPIGRAPH

Her whole life shifted the day
she started to tell the truth
about what made her happy

I never knew it could be
that simple, she said

- Kai Skye

TABLE OF CONTENTS

LIST OF ABBREVIATIONS

h: hour

d: day

N: replicate number

PSII: photosystem II

GFP: green fluorescent protein

FP: fluorescent protein

ms: millisecond

s: second

PAR: photosynthetic active radiation

PAM: pulse amplitude modulated

MQY: maximum quantum yield

EQY: effective quantum yield

F_v: variable fluorescence

F_m: maximum fluorescence

Q_m: pressure over photosystem II

F_m': maximum fluorescence for EQY

ΔF: variable fluorescence for EQY

$MgCl_2$: magnesium chloride

PPFD: photosynthetic photon flux density

SOD: superoxide dismutase

RFU: relative fluorescence units

LIST OF FIGURES

LIST OF TABLES

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ABSTRACT OF THE THESIS

Evidence of Photoenhancement by Green Fluorescent Proteins in Low Light Conditions: A Potential Factor of Habitat Distribution for the Sea Anemone *Anthopleura sola*

by

Kelly Govenar

Master of Science in Marine Biology

University of California San Diego, 2021

Professor Eric Allen, Chair

Ambient light intensity is a critical factor affecting species distribution, including the sea anemone, *Anthopleura sola*. In coastal waters, ambient light varies greatly with geographical span and within one location, for instance with increasing depth of the intertidal zone or within crevices of rocks in tidepools. *A. sola* inhabits diverse microhabitats within the subtidal and intertidal zones that are subject to differing light intensities, making it important to understand the effects of solar radiation on the sea anemone distribution in relation to its endosymbiotic zooxanthellae. A particularity of *A. sola* is it's brightly fluorescent, which could be related to the presence of the species in the supratidal, well exposed to intense sunlight, in which case the

fluorescence could be acting as a photoprotective mechanism. In this case, however, the species is also reported to cover itself with pieces of debris, thus mimicking exposure to shade rather than sunlight. Here, we postulated that the green fluorescent proteins (GFPs) of *A. sola* act as photoenhancement to redirect light to fuel photosynthesis for their endosymbiotic zooxanthellae. This was tested by investigating the native auto-fluorescence of *A. sola* in response to changes in low intensity of ambient light (to mimic shade), and to simultaneously monitor the photochemical efficiency of photosystem II of the zooxanthellae during a light controlled and flow through 106-day laboratory aquarium experiment. The experiment included an 82-day exposure period with three levels of ambient light intensities: 100% (control), 53%, and 22%, and a 24-day recovery period without light shading (100% of ambient light intensity). This research combines measurements of light intensity in the field and an experimental aquarium setting. Image analysis and an underwater fluorometer demonstrated changes in fluorescence intensity of sea anemones and the photosynthetic efficiency of zooxanthellae in response to light changes. Only the lowest light condition (22% of ambient light intensity) caused an increase in green fluorescence intensity, which suggests photoenhancement of GFPs. The endosymbiotic zooxanthellae chlorophyll maintained high efficiency of photosystem II in all light conditions and the zooxanthellae density remained unchanged; therefore, they seemed to not be affected by different light shading. The photoenhancement of *A. sola*'s GFPs in this experiment could explain *A. sola*'s ability to live and adapt to low light conditions in the intertidal and particularly the subtidal zone, explaining one factor affecting their geographical and local distribution.

Introduction

This master's thesis addresses the topic of species geographical distribution in response to adaptations to local environmental factors. Coastal environments are rich marine habitats where species can take advantage of geomorphological diversity in combination with the abundance of nutrients. Species distribution within coastal environments is determined by many factors, with solar irradiation, which influences ambient light intensity and temperature and can vary greatly with geographical span and within one location. Ambient light intensity is a critical factor affecting species distribution, especially for sessile species that reproduce sexually. *Anthopleura sola*, a solitary non-colonal sea anemone, is a useful organism to study the effects of ambient light intensities on its distribution in coastal environments.

Anthopleura sola was previously thought to be a variant of the colonal aggregating sea anemone, *A. elegantissima* (Francis, 1979), but was described as a new species by Pearse and Francis (2000). The green color of *A. sola* is due to UV absorbing pigments and is displayed as radiating lines on the oral disk (Schik and Dykens, 1984; Secord and Augustine, 2000) (Figure 1). *A. sola* inhabits lower intertidal and subtidal zones spanning thousands of miles from Northern Mexico to Bodega Bay, California (Francis, 1979), not only living in tidepools, between rocks and crevices, and on pier pilings, but also under coarse sand (Harris, 1991). The physiological and behavioral adaptations of these sea anemones help them survive in the ever changing intertidal and subtidal environments (Shick, 1991). The wide range of geographical habitats of *A. sola* and other *Anthopleura* species shows their adaptability to differing environmental conditions. *A. sola* does not reproduce via asexual binary fission like its sibling-species, *A. elegantissima*, but instead reproduces sexually via planktonic larvae, giving *A. sola* distinct genotypes (Francis, 1979). The tentacles with stinging nematocysts of *A. sola* catch

plankton, debris, and even larger food items to ingest and use for metabolic digestion (Sebens, 1977) (Figure 1). *A. sola* has another mechanism of obtaining nutrients via their endosymbiotic zooxanthellae, which is common of other *Anthopleura* species, that represents a carbon source through photosynthesis.

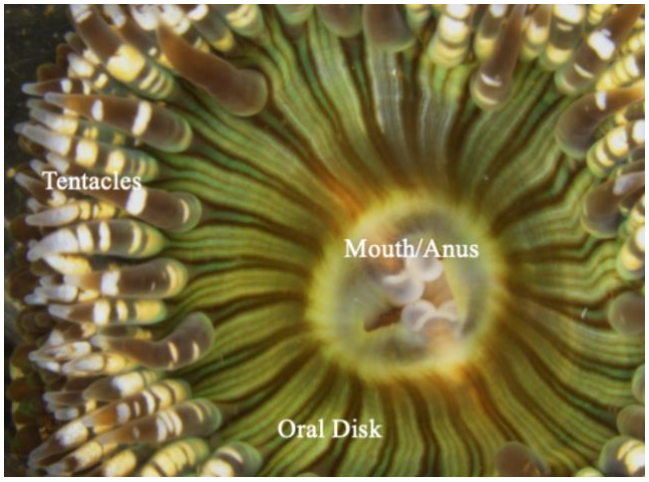

Figure 1. One of the experimental sea anemones, *A. sola*, showing the green pigmentation, and radiating lines stretching on the oral disk from the mouth/anus all the way to the tentacles. Image taken with a Nikon MODEL imaging stereoscope in brightfield reflectance (magnification: 0.5x, exposure: 1s).

The endosymbiotic golden-brown algae of *A. sola*, the *Symbiodinum* sp. zooxanthellae group common to Cnidarians (and corals in particular), are known to contribute to primary production (Secord and Augustine, 2000). The zooxanthellae are acquired from the environment, ingested, and taken up into endodermal cells of the sea anemone (Schwarz, Weis, and Potts, 2002). These zooxanthellae photosynthesize, respire, and contribute algal carbon to the host anemone at rates dependent on seasonal changes in temperature and light (Verde and McCloskey, 2007). During photosynthesis, algae convert sunlight and carbon dioxide into fixed carbon. In this symbiotic relationship between the cnidarian host and zooxanthellae, the algae provide the host with carbon while the host provides the algae inorganic carbon and nutrients

(Muscatine, 1980). At times when *A. sola* requires more nutrients, the endosymbionts supplement the host metabolism with inorganic nutrients, which provides the necessary carbon for the sea anemones to survive and adapt to seasonal and tidal changes including exposure to differing levels of solar radiation (McCloskey et al., 1996; Verde and McCloskey, 2007). The endosymbiotic relationship is therefore dynamic and more or less dependent on local environmental conditions.

Microhabitats provide protection from abiotic and biotic stressors, which seem to have determined the ecology of *A. sola* (Harris, 1991; Francis, 1979). The intertidal and subtidal zones expose *A. sola* and subsequently its symbiotic relationship to stressors including heat (from solar irradiance), variable ambient light levels, changing water currents, turbulent wave action, variable predation pressure, and intraspecific competition, especially in protected spaces (Francis, 1979; Harris, 1992; Secord and Augustine, 2000; Verde and McCloskey, 2007). Light intensity, in particular photosynthetic active radiation (PAR) and visible light, which are two components of solar radiation, is the particular interest of this thesis. Other components of solar radiation include heat (via infrared, especially at low tide) and UV (McCloskey et al., 1996; Verde and McCloskey, 2002). These factors have a limited influence, especially UV because it is rapidly absorbed by water so it can only have an effect at low tide or in very shallow waters.

Light intensity is therefore the most critical factor of solar irradiation that affects intertidal *A. sola*, which will be investigated in this thesis. Accordingly, it was shown by Verde and McCloskey (2002) that net photosynthesis of zooxanthellae and potential carbon contribution to the host relate to light intensity. Within the intertidal and subtidal zones, light intensity varies in the microhabitats in which *A. sola* inhabits; therefore, it is important to understand how this affects *A. sola*, their zooxanthellae endosymbionts, and the interaction

between the two. Solar irradiance is essential to photosynthesis, but an excess, as well as a limitation, can damage the photosynthetic system of the algae as well as the host.

A behavioral response of *A. sola* to an increase in light is the retraction of tentacles and constriction of oral disk (Pearse, 1975), which shades the zooxanthellae and reduces oxygen production. This contraction response also occurs when exposed to unfavorable stimulus (Pearse 1975). Most of the chlorophyll, and therefore the zooxanthellae, resides in the tentacles and oral disk of the host (Dykens and Shick, 1984). One reason for the expulsion of zooxanthellae in high light conditions is the production of photosynthetically generated hyperbaric O_2, an unavoidable by-product of photosynthesis, which can create oxygen toxicity for the sea anemone host. Another response to the toxic molecular oxygen production is the contraction of the sea anemone to shade the algae and maintain production of two enzymes, superoxide dismutase (SOD) and catalase, by the host proportionally to the algae chlorophyll levels (Dykens and Shick, 1984). In addition to contraction, sea anemones cover themselves with shells and debris as a "sunscreen" to decrease light exposure (Pearse, 1975).

In lower light conditions, sea anemones with zooxanthellae symbionts increase their surface area via expansion of oral disk and tentacles to maximize illumination, which favors maximum photosynthesis of zooxanthellae (Pearse, 1975). In contrast, sea anemones without zooxanthellae do not perform regular contraction and expansion in response to differing light conditions, implying this is an adaptation used by the sea anemone host to modulate light solely for the endosymbionts (Pearse, 1975). The zooxanthellae require sunlight to perform photosynthesis in order to survive; therefore, extreme low light conditions can cause mortality of the symbionts, which in turn negatively affects the sea anemone host. The death or expulsion of endosymbiotic zooxanthellae in response to unfavorable conditions, similarly to fellow

Anthozoans, hard corals, causes bleaching of the host (Brown et al., 2000), which can be coupled with increased mortality (Glynn and D'Croz, 1990).

A. sola produces fluorescent proteins, synonymous to green fluorescent proteins (GFPs), which has the potential to act as photoenhancement or photoprotection mechanisms because of their natural absorption and emission properties and based on the possible roles given to them in other organisms (mostly corals). GFPs can help with blocking damaging light. If the light is too intense, the system cannot sustain the intensity and the zooxanthellae can get photodegraded; therefore, it is assumed that GFP, which permeates the epithelium of the sea anemone, could absorb some of that light, blocking it from reaching the zooxanthellae, which would then be, as a result, exposed to lower light levels. Such sunscreen/photoabsorbing function is often also challenged by the "opposite" function, by which GFPs absorb blue damaging light and redirect it into green light that is beneficial to the zooxanthellae, thus being associated with a photoenhancement function. This function would be particularly advantageous in shady environments. There is little known about GFP modulation of sea anemones in low light conditions; therefore, this topic needs to be further explored.

GFPs were originally isolated from jellyfish (Tsien, 1998) and absorb high-energy blue light and emit lower energy green light, as green fluorescence, which can be seen in the oral disk and tentacles of *A. sola* (Shimomura et al., 1962) (Figure 2). Most of the research on biofluorescence has been done on Cnidarian corals (Shagin et al., 2004; Roth et al., 2010; Roth et al., 2013). A majority of the past research on the biology/ecology of GFP has the interest to understand the biological function (if any) of GFPs in corals, including the possibility of using the change in coral fluorescence as a proxy of stress, especially in relation to levels of ambient light. Roth et al. (2010) showed that *Acropora yongei*, a scleractinian coral, regulates GFPs to

modulate intracellular light intensities that surround the zooxanthellae, with a high GFP concentration in high light exposure, acting as a photoprotective function. In addition, Roth et al. (2010) showed the occurrence of changes in zooxanthellae density, photosynthetic pigment concentration, and photosynthetic efficiency in response to higher light conditions. The coral host and endosymbiotic dinoflagellates both exhibited a photoacclimation response in this study. Corals have also been shown to manage light with their calcium carbonate skeleton which leads to better algal growth and photosynthetic efficiency (Wangpraseurt et al, 2020). Roth and Deheyn (2013), in a cold and heat stress study, showed that green fluorescence could be an effective proxy for the health of some corals because there was a change in fluorescence that indicated stress before the onset of coral bleaching. The present work aims to test whether a similar use of change in fluorescence could be an early indicator of sea anemone health in response to changes in ambient light levels.

The emission spectrum of GFP in sea anemones peaks between 500-520 nm (green light), which results from an excitation close to 460 nm (blue light) (Johnson et al., 1962). Symbiotic zooxanthellae also fluoresce by way of absorption of light by chlorophyll and re-emission of light as chlorophyll red fluorescence, with the emission being of a longer wavelength than absorption (Maxwell and Johnson, 2000). It has been hypothesized that GFPs function as photoprotection, which is the ability of sea anemones to shield themselves and endosymbiotic zooxanthellae from UV radiation, or photoenhancement, which is the ability of GFP to transform deleterious light (UV/blue) into a more biological active wavelength (green) for use in photosynthesis by the zooxanthellae. GFPs are important for prospective photoprotection in sea anemones because they absorb potentially harmful high-energy photons of light and emit lower energy photons of light.

GFPs in sea anemones could also be produced as an antioxidant in response to stress. Sea anemones respond to stress via an oxidative reaction, which could be counter-balanced by the production of antioxidants, with GFP possibly being one of them. Palmer et al. (2009) showed that coral FPs are produced as supplemental antioxidants that could be working to prevent oxidative stress in coral tissue. Sea anemone GFPs could have this additional role of reactive oxygen species (ROS) quenching, similarly to corals.

The objective of this study was to investigate the native auto-fluorescence of *A. sola* in response to changes in ambient light intensity, and to simultaneously monitor the photochemical efficiency of photosystem II of symbiotic zooxanthellae. As discussed, *A. sola* inhabits diverse microhabitats within the subtidal and intertidal zones that are subject to differing light intensities, raising questions about the effects of solar radiation on the sea anemone fluorescence and their endosymbiotic zooxanthellae. A laboratory and light controlled flow through aquarium experiment using the sea anemone *Anthopleura sola* was used to test the hypothesis that sea anemones produce GFPs for photoenhancement to redirect light to fuel photosynthesis. This research combines measurements of light intensity in the field and an experimental aquarium setting. Image analysis and an underwater fluorometer were used to assess the changes in fluorescence intensity of sea anemones and the photosynthetic efficiency of zooxanthellae in response to light changes. The possible photoenhancement property of fluorescence in *A. sola* could potentially be used to express solar radiation stress in subtidal and intertidal zones, which could explain geographical or local distribution.

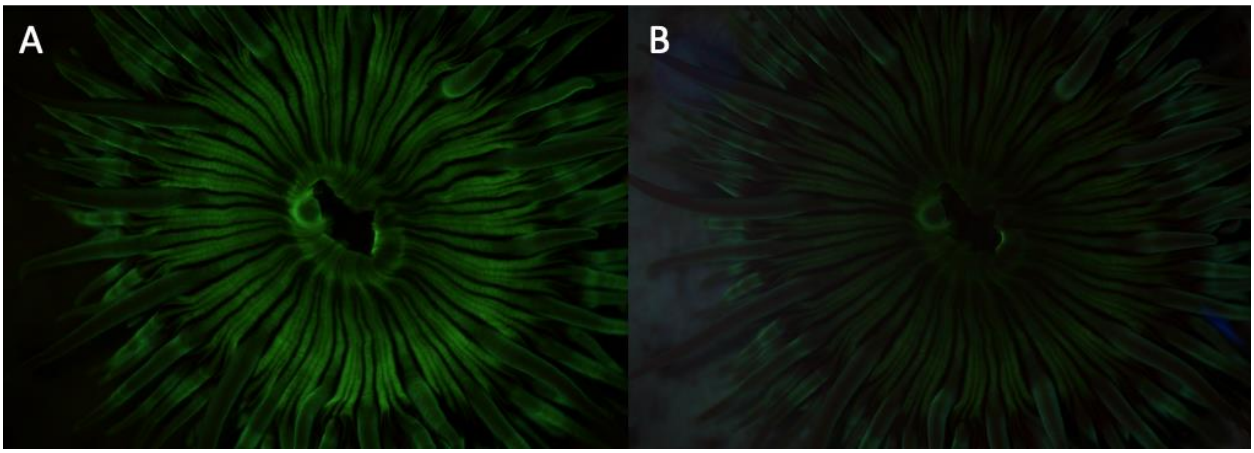

Figure 2. Biofluorescence of *A. sola* is brighter when excited in the blue/green than in the UV/blue range. Consecutive images of biofluorescence from the same individual captured under an excitation in the blue/green (470 nm) (A) and UV/blue (390 nm) (B) with exposures of 300 ms and 3s respectively, both with a magnification of 0.5x.

Materials and Methods

A two-phase photoacclimation aquaria experiment was conducted to study the effects of changes in ambient light intensity on the native auto-fluorescence of *Anthopleura sola* and the photochemical efficiency of photosystem II of their endosymbiotic zooxanthellae. Images taken with fluorescent imaging stereoscopes and microscopes were used to provide a proxy of GFP and zooxanthellae chlorophyll concentration based on levels of biofluorescence. The photochemical efficiency of the zooxanthellae chlorophyll was characterized using a Diving-PAM-II Underwater Chlorophyll Fluorometer. Photosynthetic active radiation (PAR) and ambient light intensity in the aquaria were measured with a handheld MSC15 spectral light meter and an Onset HOBO Pendant Temperature/Light 64K Data Logger respectively. The HOBO pendant was also used to measure *in situ* light intensity in local tide pools and while I scuba dived on cloudy and sunny days in shaded, partially shady, and fully exposed positions. The experimental and *in situ* light intensity measurements were compared.

Sea anemone collection

Specimens of *Anthopleura sola*, a local species of sea anemone, were collected from the Scripps Institution of Oceanography (SIO) Pier water trough in La Jolla, CA on July 10,th 2017 and kept in a tank in the SIO Hubbs Hall experimental aquarium. They were fed one scoop of AP100 Dry Larval Diet (100-150 microns) and mixed into the tank water twice a week. Starting on September 21st, 2017, the sea anemones were fed one mackerel between them once a week. The anemones were no longer fed starting two weeks prior to the start of the experiment on January 24th, 2019.

Experimental set-up

For the experiment, individual sea anemones (N=24) were each placed on 1.5 x 1.5-inch ceramic tiles within a custom-built cage system (Figure 3). Two weights were attached to the cage system and a one-inch layer of mesh was added to the bottom to prevent the escape of individuals. Sea anemones were maintained under a photoperiod of 12:12 h light:dark, to mimic the natural photoperiod, within a light controlled box with a T5-HO Fluorescence Light with two 6000K bulbs and two Aqua Blue bulbs. Each tank had one seawater inlet hose aquarium tube (diameter of 1 cm) with constant and consistent flow between tanks. Light intensity was manipulated by using plastic mesh secured to PVC pipes that fit snuggly around the top of two of the three tanks (Figure 4). The two tanks with mesh coverings were also covered on four sides with black-out plastic to prevent any light entrance other than the top of the tank (Figure 4). Prior to the start of the experiment, PAR was measured in each tank using a handheld MSC15 spectral light meter (Gigahertz-Optik GmbH), which has a spectral range of 360 nm to 830 nm. The measurements of the two lower light conditions were taken with the mesh coverings on the tanks and used to calculate the percent light of the control. Treatment 1, Treatment 2, and the control light intensities will be referred to as 22%, 53%, and 100% respectively. All three levels of light tested were considered "shade levels" as compared to field measurements (see below); therefore, it must be noted that the 100% here is relative to the experimental light level tested with additional shading and does not represent 100% of natural light levels.

A two-phase photoacclimation experiment was then conducted. In Phase I, shading was put in place on the tanks and thus exposed sea anemones to lesser light levels. In Phase II, the shading was removed and thus there was a recovery to normal experimental light levels. At the beginning of Phase I, sea anemones were placed in three ambient light treatments for a total

duration of 82 days: 22% (one layer of mesh, N=6), 53% (three layers of mesh, N=6), and 100% (no mesh, N=12 control) of ambient light. At the beginning of Phase II (starting at 83 d), the mesh was removed, and all sea anemones were returned to 100% ambient light intensity for a recovery period (83-106 d). Throughout both phases, every seven days, sea anemones (0 d, 1 d, 82 d, 106 d: N=24, 7-106 d: N=6 per treatment) were removed from the tanks and imaged for green fluorescence and chlorophyll fluorescence intensities. During Phase I and II, the endosymbiotic zooxanthellae were measured for maximum quantum yield of photosystem II (0 d, 2 d, 6 d, 9 d, 13 d, 16 d, 20 d, 23 d, 27 d, 30 d, 34 d, 44 d, 51 d, 58 d, 65 d, 72 d, 79 d, 86 d: N=24).

Figure 3: The cage system that was put around each sea anemone to contain each of the individuals.

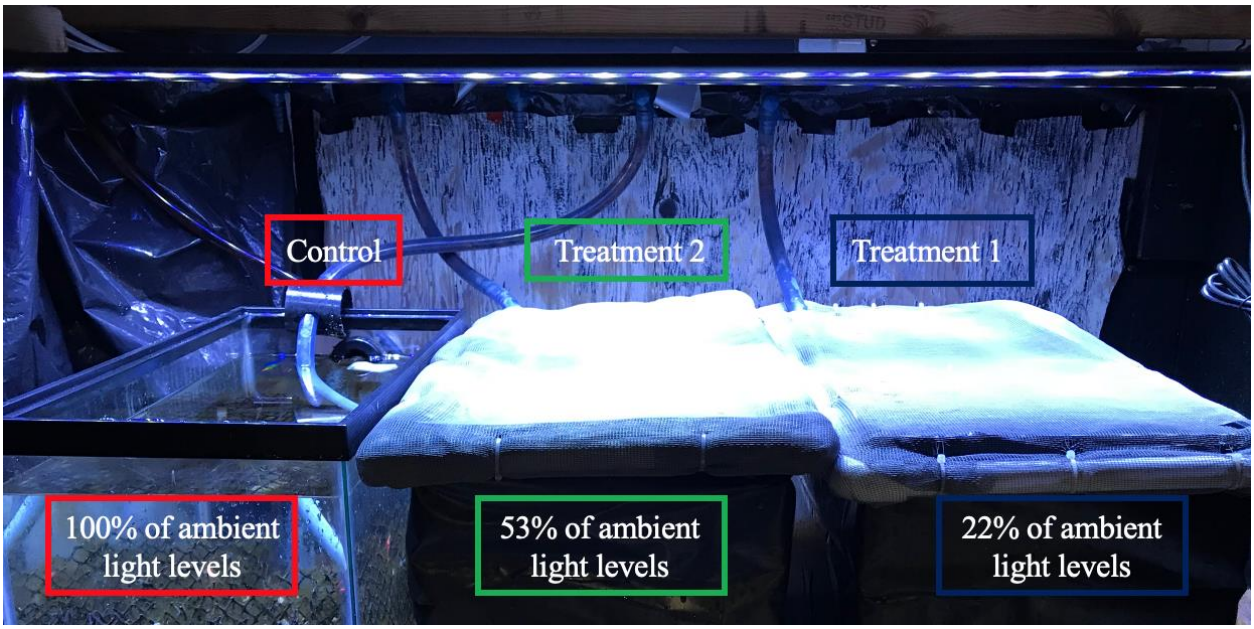

Figure 4: Image of the experimental set-up with three tanks: control (100% of ambient light levels - no mesh), Treatment 1 (22% of ambient light levels- three layers of mesh), and Treatment 2 (53% of ambient light levels - one layer of mesh).

Green and red fluorescence imaging intensity

This was used to provide a proxy of GFP and chlorophyll (zooxanthellae) concentration based on levels of biofluorescence from images (GFP in the green range vs. chlorophyll in the red range). To determine green and red fluorescence intensity, during both phases of the experiment (1 d, 8 d, 15 d, 22 d, 29 d, 36 d, 43 d, 50 d, 57 d, 64 d, 71 d, 78 d, 85 d, 92 d, 99 d: N=18, 0 d, 82 d and 106 d: N=24), sea anemones were imaged with a fluorescent imaging stereoscope (Nikon SMZ 1500 always with 100 W mercury lamp and filter cube with excitation 390 nm and 470 nm) under the same settings, which included the exposures 300ms, 600ms, 900ms, 2s, 5s, and 7s for the excitation of 390 nm, the exposures 20ms, 60ms, 100 ms, 300ms, 600ms, 900ms for the excitation of 470 nm, and consistent 0.5x magnification. In addition, the same field was photographed under white light (in reflectance) with the exposures 40ms, 80ms, 200ms, 500ms, 700ms, and 2s. Prior to photographing, the stereoscope was standardized with an

FRS-5 NightSea. The sea anemones were anesthetized (8% $MgCl_2$ in seawater) during the photographing process for ten minutes and returned to seawater immediately following to recover.

The images taken with an exposure of 300ms with an excitation of 470 nm (Figure 5) and an exposure of 5s with an excitation of 390 nm were processed in Fiji 1.0 (ImageJ) to obtain the average green and red fluorescence intensities, respectively. The 470 nm and 390 nm wavelengths were used as a proxy for GFP and chlorophyll respectively; therefore, it is necessary to use both spectra. Images were split into three color channels: red (red fluorescence), green (green fluorescence), and blue. The blue channels were not used. For each image processed, ten measurements, with an area of 1264 pixels, of fluorescence intensity were taken around the oral disk area of the sea anemone and averaged to get the average fluorescence intensity.

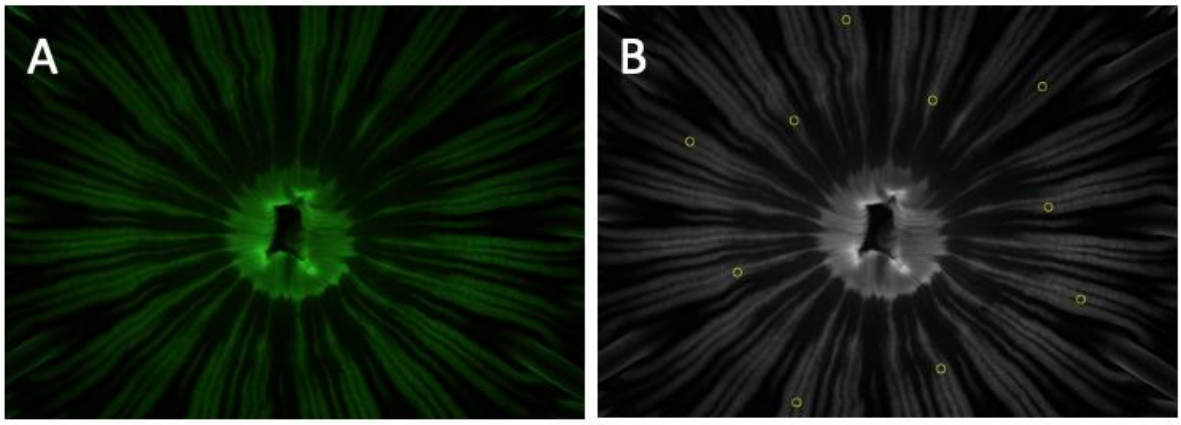

Figure 5. Image (A) of the oral disk of one sea anemones from the 22% of ambient light on Day 0 taken with a fluorescent imaging stereoscope with a UV/Blue excitation of 470 nm and exposure of 300 ms. Green (green fluorescence) color channel image (B) split in Fiji (from image A) with ten areas selected to find the average fluorescence intensity.

Photochemical efficiency of photosystem II

This technique was used to characterize the level of photosynthesis by the zooxanthellae chlorophyll. An Underwater Diving-PAM-II Chlorophyll Fluorometer from Heinz Walz GmbH was used to assess the photoacclimation and physiological status of the zooxanthellae (0 d, 1 d, 2 d, 6 d, 9 d, 13 d, 16 d, 20 d, 23 d, 27 d, 30 d, 34 d, 44 d, 51 d, 58 d, 65 d, 72 d, 79 d, 86 d: N=24). From 87-106 d, PAM measurements failed due to PAM malfunctions. A 0.5 by 0.5-inch sponge square was cut with a hole in the center to fit snugly around the end of the probe to create consistent distance from the probe to the center of the sea anemones for measurements. Sea anemones were anesthetized for the measurements and returned to seawater immediately following to recover. The probe with the attached sponge was gently pressed to the center of the anemone for the measurements. The experimental aquarium was completely shielded from extraneous light. The dark-acclimated maximum quantum yield (MQY) of PSII (F_v/F_m; F_v, variable fluorescence; F_m, maximum fluorescence) was measured pre-dawn in the dark to prevent zooxanthellae exposure to any light, especially blue/green light. The light-acclimated effective quantum yield (EQY) of PSII ($\Delta F/F_m$') was measured at the sea anemones' mid-day (Warner et al., 1996) six hours after the aquarium lights turned on when the sea anemones were light acclimated. The pressure over PSII was determined as $Q_m = 1 - [(\Delta F/F_m$' at mid-day) / (F_v/F_m at pre-dawn)] to compare pre-dawn and mid-day quantum yields. Measurements were conducted pre-dawn and mid-day to see how much the photosystem II of the zooxanthellae chlorophyll could recover from any photodamage.

Light intensity

The ambient light intensity was compared for the lab aquaria and the environment. I studied field intensities in local tide pools and while scuba diving. Relative light intensity measurements were taken every 30 minutes throughout the entire experiment using an Onset HOBO Pendant Temperature/Light 64K Data Logger attached to the surface of each tank. The HOBO Pendant had a spectral range of 150 nm to 1200 nm.

To relatively compare light intensity experimental conditions to *in situ* conditions, light intensity was measured in three tidepools at the Dyke Rock tidepools, just north of Scripps Institution of Oceanography, during low tide (Figure 6). The Onset HOBO Pendant Temperature/Light 64K Data Logger was placed in three positions in each tidepool: fully exposed, partially exposed, and shaded, which were located directly next to each tide pool, at the bottom of each tidepool (about 6 inches deep), and under a shaded rock respectively. The HOBO Pendant recorded light intensity every 10 seconds continuously for three minutes at each location on July 22nd and August 5th, 2020, a cloudy and sunny day, respectively.

In addition, relative light intensity measurements were taken with the Onset HOBO Pendant Temperature/Light 64K Data Logger off of the Scripps Pier in La Jolla, CA. While I scuba dived, the pendant logged light intensity every ten seconds for three minutes at each location on August 13th and September 30th, 2020, a cloudy and sunny day, respectively. Measurements were taken at the surface (0 feet), midwater (~10 feet), and bottom (~20 feet) at three locations near or under the pier which were locations fully exposed, partially exposed, and shaded in regard to sunlight exposure (Figure 7).

Figure 6. Image of the tidepools at Dyke Rock in La Jolla, CA. HOBO Pendant was used for light intensity measurements in the positions: (1) shaded (under a rock), (2) partially exposed (bottom of the tide pool), and (3) fully exposed (side of tide pool).

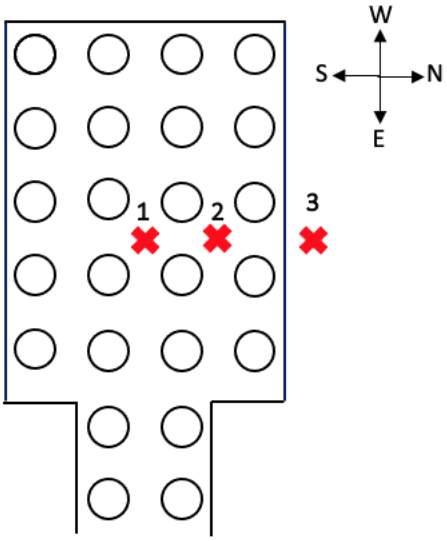

Figure 7. An illustration of the aerial view of the far west portion of the Scripps Institution of Oceanography pier pilings in La Jolla, CA. The red X's represent the location of light intensity measurements taken with the HOBO Pendant Data Logger. The sunlight exposures are the locations are (1) shaded, (2) partially exposed, and (3) fully exposed.

Statistical Analysis

Statistical analyses were performed in Prism 9 (GraphPad Software, LLC). For fluorescence, PAM measurements, and light intensities, the Shapiro-Wilk test tested for normality followed by a one-way ANOVA. A Tukey's multiple comparisons test was performed when the one-way ANOVA demonstrated significant differences between treatments, as a post-hoc test. The red and green fluorescence data were transformed into a logarithmic scale to pass the Shapiro-Wilk normality test (Whitlock and Schluter, 2009). For the red fluorescence and effective quantum yield data, seven outliers were removed (Q=5%) and one outlier was removed (Q=1%) respectively to adhere to normality.

Results

Light intensity variation in aquaria and in the field

Before the start of the experiment, the percent light of the control was found to be 100% (control), 53% (Treatment 2), and 22% (Treatment 1) using photosynthetic active radiation (PAR) measurements with the results shown in Table 1. Light intensity measured with the HOBO pendant monitored the consistency of the light intensities from day to day and confirmed the light intensities did not change over the entire experiment within each tank. The relative light intensity measurements taken during the experiment with the HOBO pendant are shown in Figure 8. The PAR (Table 1) and light intensity (Figure 8) measurements cannot be compared directly because they measure light in different spectral ranges; however, light intensity encompasses PAR.

In the tidepools, the light intensity decreased with decreasing exposure (Figure 9). The fully exposed, partially exposed, and shaded positions in the tidepools differed significantly between the cloudy and sunny days (ANOVA: $p < 0.05$), with the sunny days having significantly higher light intensities (Figure 9). The light intensity depth profiles measured during scuba dives revealed a decreasing light intensity with increasing depth and exposure level on both cloudy and sunny days (Figure 10). The highest light intensity can be seen in the fully exposed position in the tidepools on the sunny day with an average of 9,230.01 ± 1,342.22 (standard deviation) lum*ft^2 (Figure 9).

The light intensity measured during the experiment are representative of low light locations in the subtidal zone. The 22% of ambient light intensity treatment showed the most similar measurements as taken on the cloudy day under the partially exposed positions at 10 and 20 ft (Figure 11). The 53% of ambient light intensity treatment showed the most similar

measurements as taken on the cloudy day under the shaded condition at 0 ft (Figure 11). The 100% of ambient light intensity corresponded well with intensities measured on the cloudy day under the fully exposed condition at 20 ft as well as on the sunny day under the shaded condition at 10 ft (Figure 11). These data show that the experimental light intensity conditions represent actual light intensities that subtidal *A. sola* experience *in situ*, but not under strong direct sunlight exposure.

Table 1. PAR values of Treatment 1 (22% of ambient light - 3 layers of mesh), Treatment 2 (53% of ambient light – 1 layer of mesh), and the control (100% of ambient light – no mesh) with standard deviations and percent light of the control taken prior to the start of the experiment in aquaria.

Treatment	PAR (N=6) (Photosynthetic photon flux density) ± SD	% Ambient Light
Treatment 1	23.45 ± 0.06	21.66
Treatment 2	57.80 ± 0.58	53.39
Control	108.27 ± 0.12	100

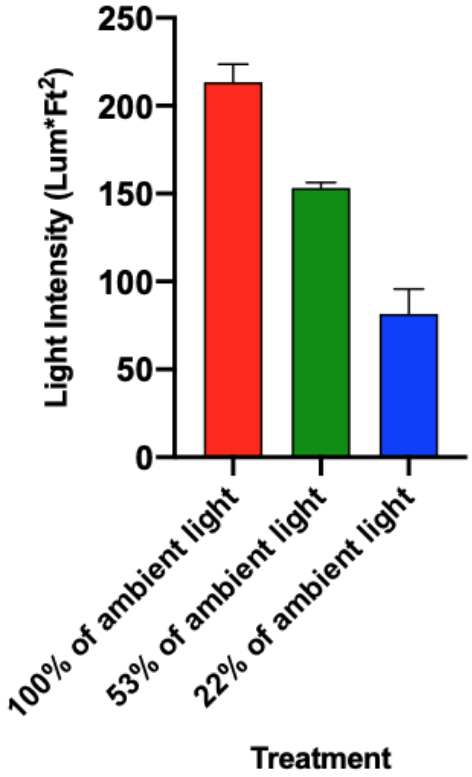

Figure 8. Light intensity measurements taken during the first week of the experiment of the 100% of ambient light (red), 53% of ambient light (green), and 22% of ambient light (blue) treatments with HOBO pendants (ANOVA: $p < 0.05$, $R^2 = 0.97$).

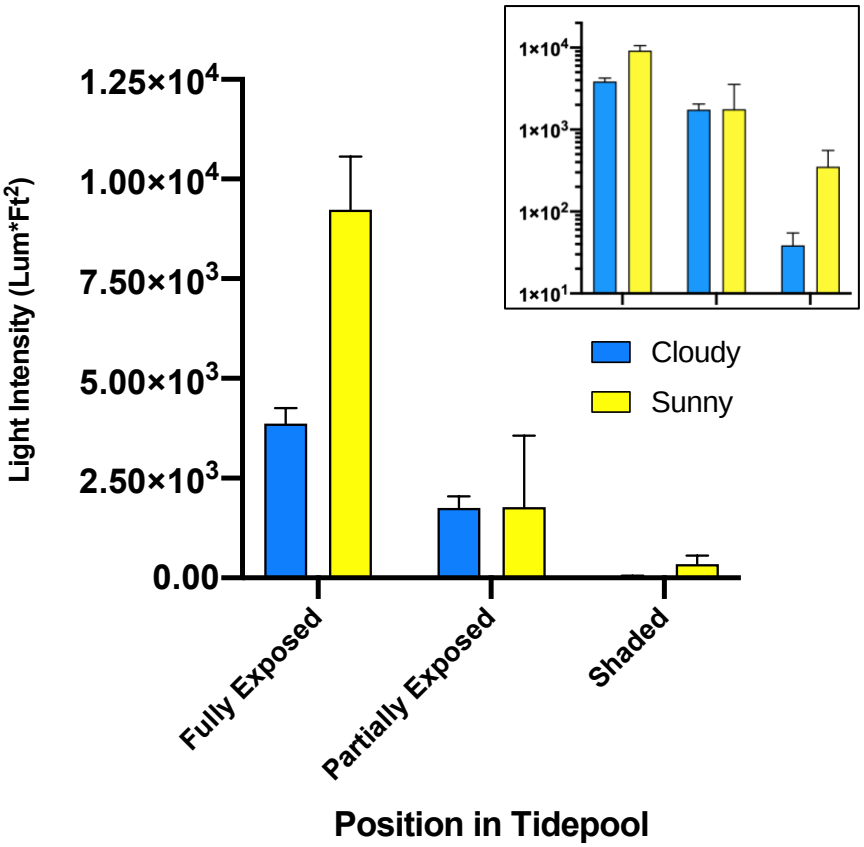

Figure 9. A grouped bar graph of light intensity measurements averaged from three tidepools at the Dyke Rock tidepools in La Jolla, CA with standard deviations. Measurements were taken at fully exposed, partially exposed, and shaded positions in the tidepools on one cloudy (blue) and one sunny (yellow) day. The average of the lowest light position (shaded) for the cloudy day was 38.63 lum*ft^2 with a standard deviation of 16.27 lum*ft^2. Insert is the log scale of the figure.

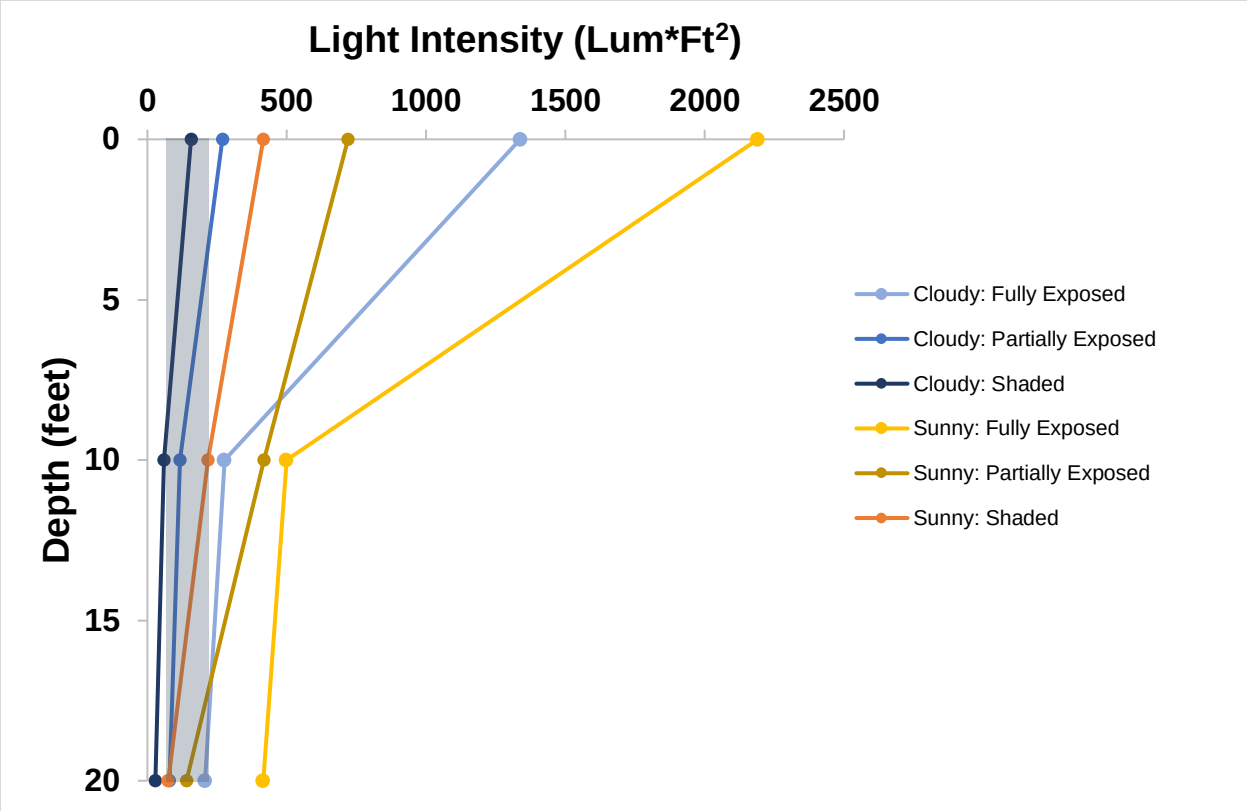

Figure 10. A depth profile of light intensity taken while scuba diving at the end of the Scripps Institution of Oceanography pier on a cloudy day (surface temperature 64.2 ºF) and sunny day (surface temperature 70.12 ºF) at three positions: fully exposed, partially exposed, and shaded. The grey shaded region represents the light intensity of the experimental conditions.

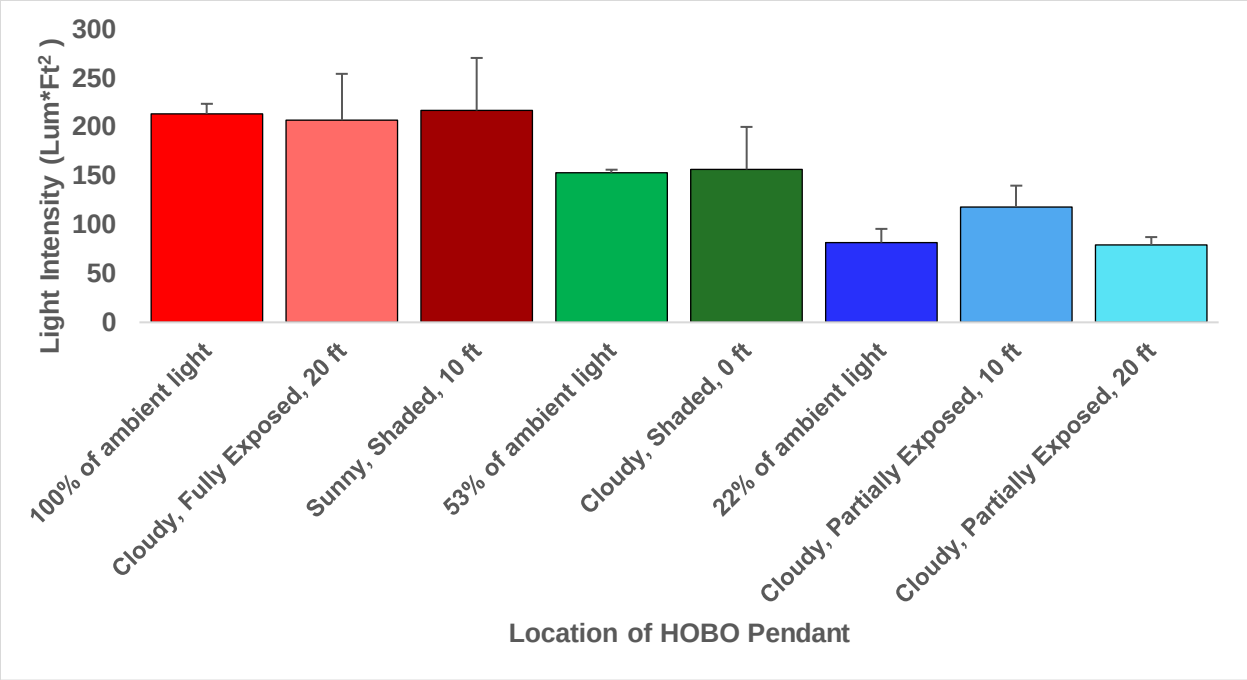

Figure 11. Bar graph showing the similar light intensities with standard deviations between specific diving sites and experimental measurements. 100% of ambient light, cloudy, fully exposed, 20 ft, and sunny, shaded, 10 ft measurements show similarities (red color tones). 53% of ambient light and the cloudy, shaded, 0 ft measurements show similarities (green color tones). 22% of ambient light, the cloudy, partially exposed, 10 ft, and the cloudy, partially exposed, 20 ft measurements (blue color tones) show similarities.

Green and red fluorescence intensity

Phase I of the experiment revealed significantly different green fluorescence pixel intensities among means of treatments (ANOVA: $p < 0.05$) (Figure 12) (Table 2). The 22% of ambient light treatment was on average 1.23 times greater than the 53% and 100% and the 100% and 53% of ambient light were not significantly different (Tukey: $p < 0.05$ A-B, $p < 0.05$ A-C, $p < 0.05$ B-C). Phase II of the experiment found no significant difference in green fluorescence pixel intensities among means of treatments (ANOVA: $p = 0.18$) (Figure 13) (Table 2).

Phase I of the experiment showed a significant difference in red fluorescence intensity between the 22% and 100% of ambient light treatments but none between the 22% and 53% of

ambient light treatments (ANOVA: $p < 0.05$; Tukey: $p = 0.27$ A-B, $p = 0.007$ A-C, $p = 0.23$ B-C) (Figure 14) (Table 2). On average, the red fluorescence pixel intensity of the 22% of ambient light treatment was 1.13 times greater than the 100% during Phase I. Phase II of the experiment showed no significant difference in red fluorescence intensity between the treatments and control (ANOVA: $p > 0.05$) (Figure 15).

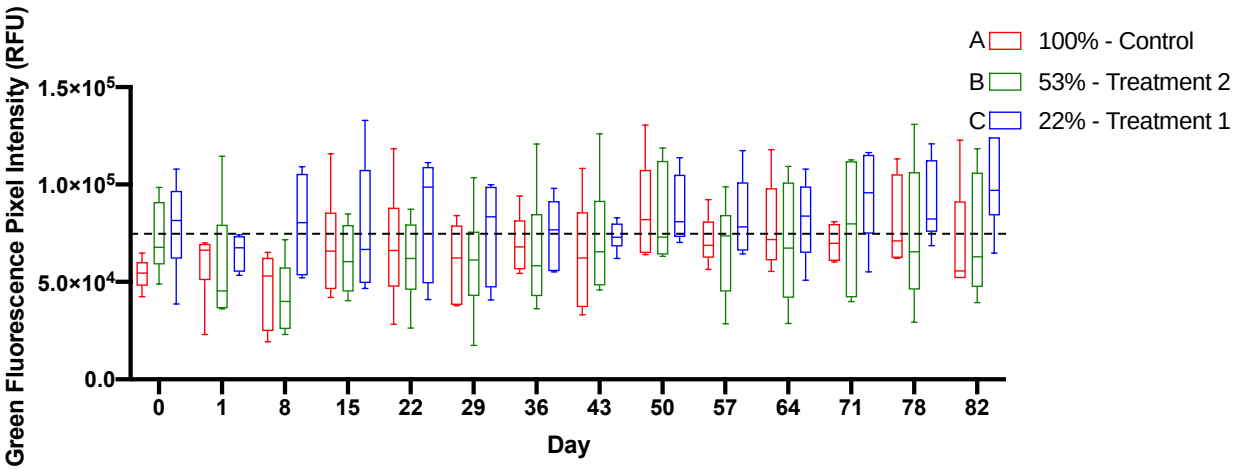

Figure 12: Box plot of Phase I mean green fluorescence pixel intensity. 100%, 53%, and 22% of ambient light intensity are labeled A, B, and C respectively. The dashed line represents the average overall (y = 72,221.79 RFU).

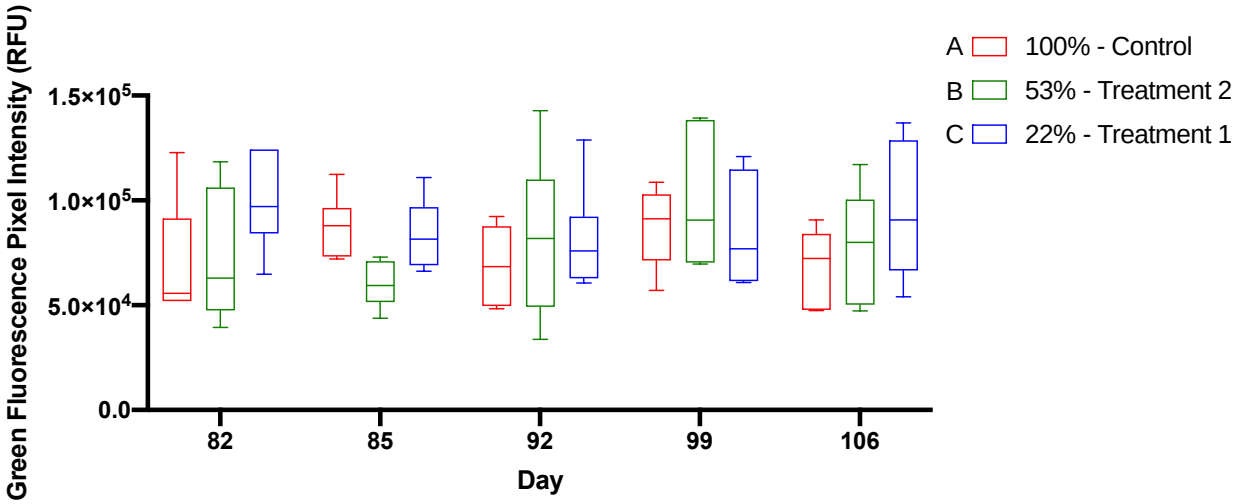

Figure 13: Box plot analysis of Phase II mean green fluorescence intensity. 100%, 53%, and 22% of ambient light intensity are labeled A, B, and C respectively.

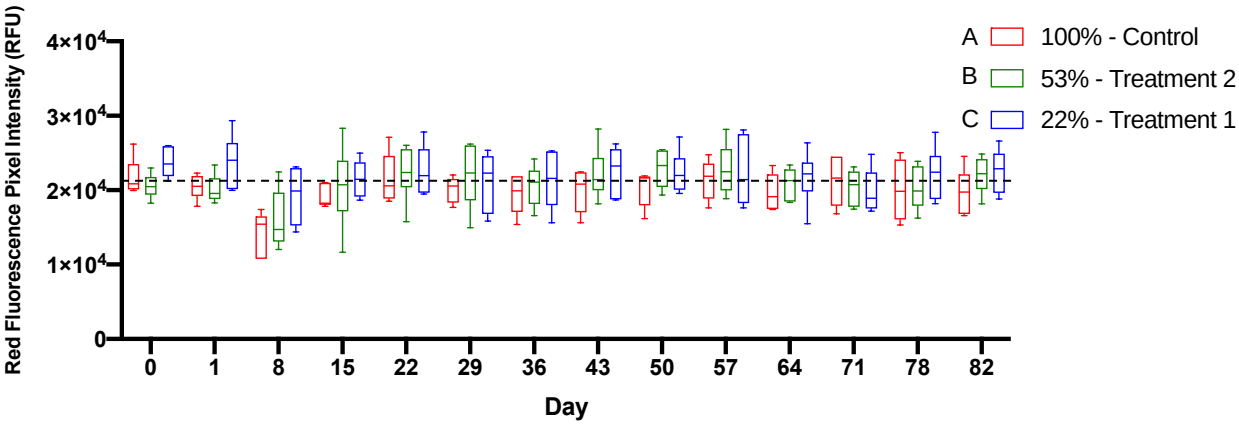

Figure 14: Box plot analysis of Phase I mean red fluorescence pixel intensity. 100%, 53%, and 22% of ambient light intensity are labeled A, B, and C respectively. The dashed line represents the average overall (y = 21,000.79 RFU).

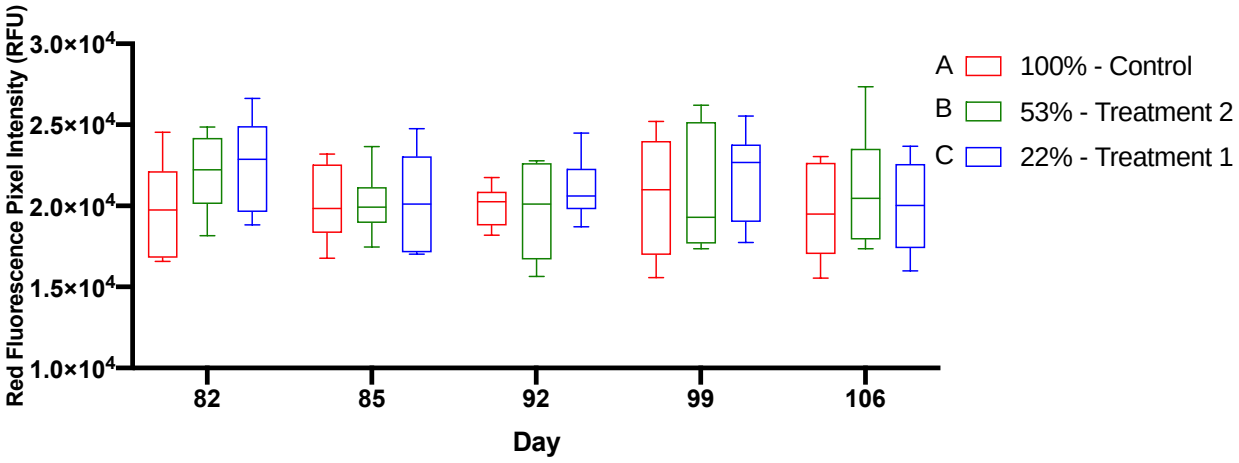

Figure 15: Box plot analysis of Phase II mean red fluorescence pixel intensity. 100%, 53%, and 22% of ambient light intensity are labeled A, B, and C respectively.

Table 2. Summary of fluorescence pixel intensity analysis of variance (ANOVA) statistics to test differences among treatment groups for individual days of Phase I and II. Tukey's multiple comparison test P value results are displayed when ANOVA P<0.05. 100%, 53%, and 22% of ambient light intensities are represented by A, B, and C respectively. Data was log transformed for statistical analysis.

Parameter			ANOVA		Tukey's Multiple Comparison Test (P)		
	Phase	Day	F statistic	P	A-B	A-C	B-C
Green fluorescence pixel intensity	I	0	2.69	0.10			
		1	0.23	0.80			
		8	5.94	0.01	0.94	0.03	0.02
		15	0.49	0.62			
		22	1.17	0.34			
		29	0.79	0.47			
		36	0.31	0.74			
		43	0.29	0.75			
		50	0.04	0.96			
		57	1.06	0.37			
		64	0.39	0.68			
		71	1.44	0.27			
		78	0.57	0.58			
		82	2.13	0.13			
	II	85	7.48	0.01	0.01	0.89	0.02
		92	0.29	0.75			
		99	0.49	0.62			
		106	1.20	0.33			
Red fluorescence pixel intensity	I	0	3.91	0.04	0.55	0.24	0.04
		1	4.06	0.04			
		8	2.53	0.12			
		15	0.47	0.64			
		22	0.14	0.87			
		29	0.26	0.78			
		36	0.46	0.64			
		43	1.08	0.37			
		50	1.68	0.22			
		57	0.21	0.81			
		64	0.68	0.52			
		71	0.35	0.71			
		78	0.68	0.52			
		82	1.76	0.21			
	II	85	*	***			
		92	0.62	0.55			
		99	0.28	0.76			
		106	0.30	0.75			

* F < 0.001
**R squared < 0.001
***P > 0.9

Photochemical efficiency of photosystem II

Dark-acclimated maximum quantum yield

From 0-86 d, there was no significant difference between the means of the treatments and the control of the dark-acclimated maximum quantum yield (ANOVA: $p = 0.33$, $R^2 = 0.04$) (Figure 16) (Table 3). There was no significant variation occurring in MQY from 0-86 d and therefore, no effect on MQY caused by the shading of light.

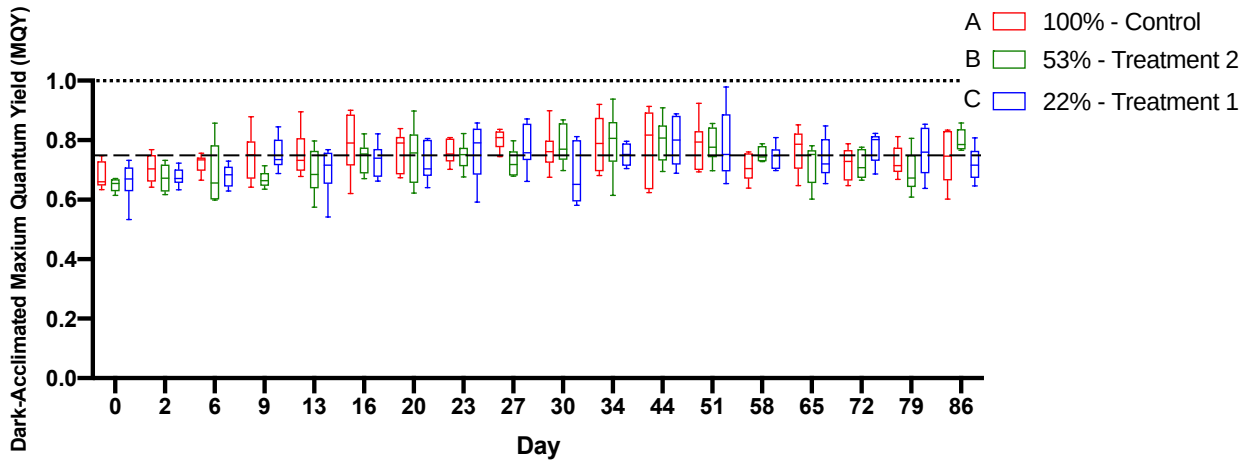

Figure 16. The dark-acclimated maximum quantum yield (F_v/F_m at pre-dawn) for 0-86 d. The dotted line represents the maximum measurement of MQY possible (y = 1). 100%, 53%, and 22% of ambient light intensity are labeled A, B, and C respectively. The dashed line represents the overall median (y = 0.743).

Light-acclimated effective quantum yield

There was no significant effect on the treatments from 0-86 d, on the light-acclimated effective quantum yield (ANOVA: $p = 0.59$, $R^2 = 0.02$) (Figure 17). However, when examining individual days, there was a significant difference in means for the light-acclimated effective quantum yield on 6 d, 9 d, 16 d, 30 d, and 34 d (Table 3). Thus, some variation occurred during Phase I of the experiment but with no definitive trend or consistency. For the 22% of ambient

light treatment, the effective quantum yield on 9 d reached a maximum followed by a decrease on 13 d and a minimum on 16 d. On 21 d, the effective quantum yield returned to close to initial levels (Figure 17).

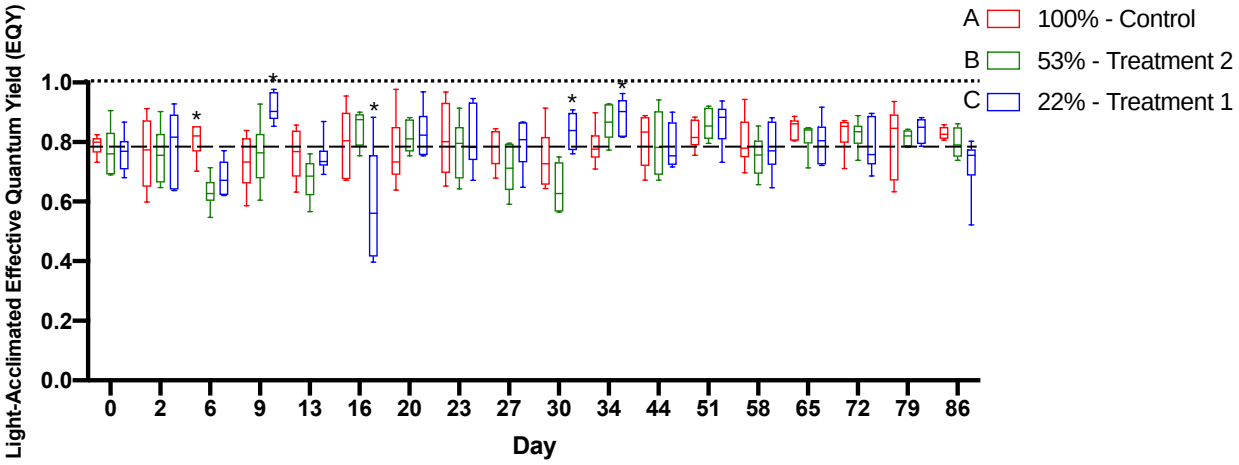

Figure 17. The light-acclimated effective quantum yield ($\Delta F/F_m$' at mid-day) for 0-86 d. The dotted line represents the maximum measurement of EQY possible (y = 1). 100%, 53%, and 22% of ambient light intensity are labeled A, B, and C respectively. The asterisk (*) over particular treatments indicates that treatment is significantly different from the other treatments on that specific day. The dashed line represents the overall median (y = 0.795).

Pressure over photosystem II

The Q_m measurements for treatments and control were not significantly different from each other (ANOVA: $p > 0.05$) (Figure 18). There was no trend overall in Q_m; however, some variations occurred with significance on 6 d, 16 d, 30 d, and 70 d (Table 3). Both negative (MQY < EQY) and positive (MQY > EQY) Q_m values are seen in all treatments over the course of the experiment (Figure 18).

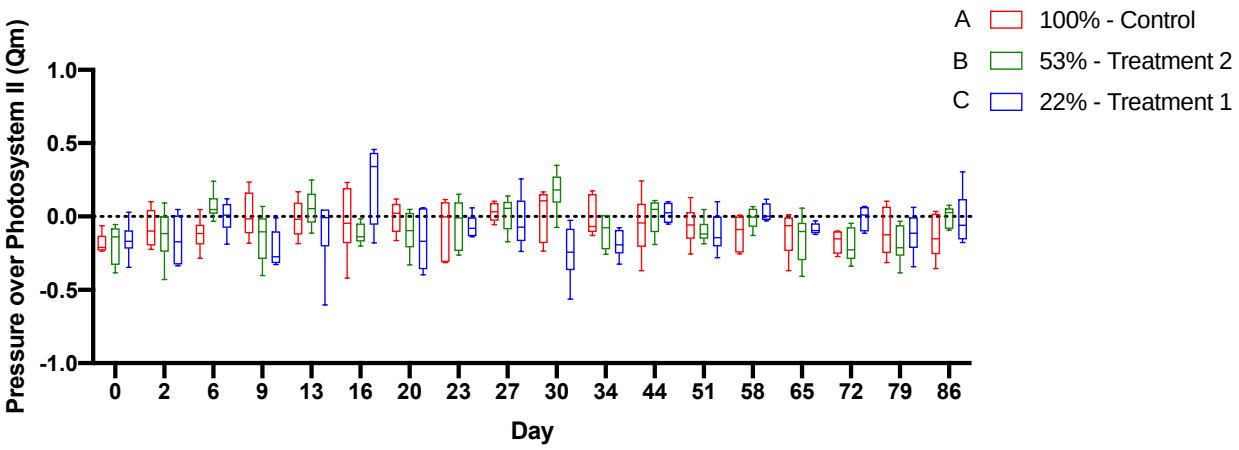

Figure 18. The pressure over photosystem II for 0-86 d calculated as $Q_m = 1 - [(\Delta F/F_m' \text{ at mid-day}) / (F_v/F_m \text{ at pre-dawn})]$. The dotted line (at y = 0) shows when $\Delta F/F_m'$ at mid-day (EQY) is equal to F_v/F_m at pre-dawn (MQY). 100%, 53%, and 22% of ambient light intensity are labeled A, B, and C respectively.

Table 3. Summary of MQY, EQY, and Q_m analysis of variance (ANOVA) statistics to test differences among treatment groups for individual days of Phase I and II. Tukey's multiple comparison test P value results are displayed when ANOVA P<0.05. 100%, 53%, and 22% of ambient light intensities are represented by A, B, and C respectively.

Parameter	Phase	Day	ANOVA			Tukey's Multiple Comparison Test (P)		
			F statistic	P	R squared	A-B	A-C	B-C
Dark-acclimated maximum quantum yield	I	0	0.59	0.57	0.07			
		2	1.05	0.38	0.12			
		6	0.71	0.51	0.09			
		9	3.73	*	0.33	0.10	0.97	0.06
		13	1.08	0.36	0.13			
		16	0.94	0.41	0.11			
		20	0.39	0.68	0.05			
		23	0.09	0.91	0.01			
		27	3.07	0.08	0.29			
		30	2.77	0.09	0.27			
		34	0.46	0.64	0.06			
		44	0.05	0.95	0.01			
		51	**	***	****			
		58	1.29	0.31	0.18			
		65	0.71	0.51	0.09			
		72	2.58	0.11	0.27			
		79	1.51	0.25	0.17			
	II	86	2.30	0.14	0.25			
Light-acclimated effective quantum yield	I	0	0.28	0.76	0.04			
		2	0.12	0.89	0.02			
		6	15.31	*	0.67	*	*	0.32
		9	7.98	*	0.52	0.83	0.01	0.02
		13	2.43	0.12	0.24			
		16	6.50	0.01	0.48	0.78	0.04	0.01
		20	0.93	0.42	0.11			
		23	0.18	0.84	0.02			
		27	2.05	0.16	0.21			
		30	8.45	*	0.52	0.12	0.15	*
		34	3.61	0.03	0.38	0.10	0.03	0.78
		44	0.10	0.91	0.01			
		51	0.81	0.47	0.10			
		58	0.55	0.59	0.07			
		65	1.10	0.36	0.13			
		72	0.77	0.48	0.09			
		79	0.37	0.69	0.05			
	II	86	3.48	0.06	0.33			
Pressure over photosystem II (Qmax)	I	0	0.09	0.91	0.01			
		2	0.41	0.67	0.05			
		6	5.13	0.02	0.41	0.02	0.16	0.46
		9	3.66	0.06	0.33			
		13	1.14	0.35	0.13			
		16	3.87	0.05	0.37	0.75	0.13	0.04
		20	1.68	0.22	0.18			
		23	0.04	0.96	**			
		27	0.44	0.65	0.06			
		30	9.37	*	0.56	0.31	0.04	*
		34	3.68	0.05	0.33			
		44	0.42	0.67	0.06			
		51	0.34	0.71	0.04			
		58	2.96	0.09	0.33			
		65	0.38	0.69	0.05			
		72	4.82	0.03	0.47	0.95	0.08	0.04
		79	0.60	0.56	0.07			
	II	86	1.27	0.31	0.15			

* P < 0.01
**F < 0.001
***P > 0.9 ****R squared < 0.001

Discussion

This study analyzed the effect of low light intensity conditions (shading) on the native auto-fluorescence of *A. sola* and the photochemical efficiency of photosystem II of their endosymbiotic zooxanthellae. The production of GFPs by *A. sola* for photoenhancement to concentrate light for zooxanthellae photosynthesis was investigated. The light intensity treatments in the experiment represented low light conditions in the field that *A. sola* experiences. The results showed that the highest amount of shading (22% of ambient light intensity) caused an increase of green fluorescence intensity and therefore, GFPs, indicating a possible photoenhancement property of GFPs in *A. sola*. The endosymbiotic zooxanthellae exhibited relatively constant levels of chlorophyll and efficiency of PSII of zooxanthellae chlorophyll suggesting the shading of light did not negatively affect the endosymbiotic zooxanthellae. The modulation of GFPs in the lowest light condition in the experiment suggests the possible photoenhancement property of GFPs, which could partly explain the local distribution of *A. sola* in the subtidal zone with low solar radiance, which is usually associated with stress for similar organisms relying on photosynthesis in coastal waters.

Light intensity in aquaria is representative of low light conditions in the field

The light conditions in the experiment (22%, 53%, and 100% of ambient light intensity) represented low light conditions *A. sola* face in the subtidal zone: 10-20 feet deep low sunlight exposure pier piling habitats, low sunlight exposure in intertidal zone, and high light exposure 10-20 feet deep subtidal and pier piling habitats respectively. These results show that the experimental conditions represent the light exposure conditions *A. sola* experience *in situ*;

however, these sea anemones in the environment are subject to an even wider range of light levels, particularly in the intertidal zone.

PAR is the amount of light available for photosynthesis, which is in the 400 to 700 nm wavelength range. It was important to base percent light levels of the treatments off of PAR because this gives a more accurate understanding of different light effects on the photosynthetic efficiency of the zooxanthellae. PAR and light intensities taken with the HOBO pendant cannot be compared because the HOBO pendant is only meant to measure relative light intensity to compare different locations/conditions of light intensity. In addition, the two measurements have a different wavelength range.

Green fluorescence intensity is only affected by largest amount of shading

The significant difference between the green fluorescence pixel intensity of the 22% of ambient light intensity treatment and the other treatments during Phase I of the experiment suggests there was a possible stress response occurring in the lowest light condition (22% of ambient light), with a PAR of 23.45 photosynthetic photon flux density (PPFD). This could imply a photoenhancement response of the sea anemone GFPs to concentrate light for algae photosynthesis only in the 22% of ambient light condition. The lack of significant changes in green fluorescence intensity for the 53% and 100% of ambient light intensity treatments suggests the higher PAR values (57.80 and 108.27 PPFD) for these treatments did not cause a stress response and *A. sola* is able to withstand these PAR values without adjusting green fluorescence intensity and therefore, the photoenhancement of GFPs is not used in the 53% and 100% of ambient light intensity. These results imply that only in more extreme low solar radiance exposure environments, the subtidal zone in particular, the sea anemone GFPs are used as

photoenhancement to concentrate light for the photosynthesis of endosymbiotic zooxanthellae. Similarly, in corals, Smith et al. (2017) found that GFP-like protein expression of the coral host photoconvertible fluorescent proteins act as a photoenhancement in low light intensity environments and function by transforming wavelengths into being readily absorbed by their endosymbiotic zooxanthellae photosynthetic pigments. This study and the present study show the importance of GFP-like and GFP protein expression as ecological mechanisms that the coral *Symbiodinium* and sea anemone, *A. sola,* use to support their inhabitance in low light environments.

Chlorophyll levels and density of zooxanthellae are not affected by shading

The red fluorescence pixel intensity of the 22% and 100% (control) of ambient light intensity did significantly differ during Phase I; however, the lack of significant difference between the 22% and 53% of ambient light intensity, and 53% and 100% of ambient light intensity implies that the density of zooxanthellae did not change over the entire experiment, since red fluorescence is mainly produced by the zooxanthellae. In addition, since the zooxanthellae did not increase chlorophyll content in response to low-light conditions, they may not need to modulate chlorophyll to fix the same amount of carbon as the high light control treatment. This is consistent with Verde and McCloskey's findings (2002) that light intensity did not significantly influence algal density of zooxanthellae in *A. elagantissima* during their short-term 10-12 day experiments. This suggests that even on longer time scales, the present experiment 106 days, ambient and synthetic light intensities do not seem to influence algal densities. Saunders and Muller-Parker (1997) found conflicting results in that the zooxanthellae of *A. elagantissima,* in which there was a steady increase in density of algae over 25 days at two

light intensities; however, they were measuring zooxanthellae densities only in the tentacles. In comparison, the present study was looking at red fluorescence, therefore chlorophyll, around the oral disk of *A. sola* and Verde and McCloskey (2002) reported algal densities of the entire organism. Both studies were looking at *A. elagantissima* while the present study was investigating *A. sola*; however, it is beneficial to make comparisons with the sibling-species (*A. elagantissima*) since they both contain endosymbiotic zooxanthellae.

The relatively consistent and steady red fluorescence intensity over time and between light exposures implies a lack of expulsion and absorption of algal symbionts regardless of light level or there is expulsion occurring at the same time as a higher growth rate of zooxanthellae. Corals with symbiotic zooxanthellae expel zooxanthellae in unfavorable conditions and in turn bleach (Brown et al., 2000). The results of the present study imply there was a lack of expulsion of zooxanthellae, suggesting that the low light conditions were not unfavorable enough to cause the release of zooxanthellae and therefore, bleaching. In future studies, algae expulsion, density, and growth should be measured using methods described by McCloskey et al. (1996) to more definitively know how the zooxanthellae react to different light intensities by looking at these factors. Since *A. sola* inhabits a wide range of habitats, in the subtidal and intertidal zones, the zooxanthellae and sea anemone host may be adapted to withstand the range of light intensities tested in this experiment and found in the natural environment.

Zooxanthellae chlorophyll capable of high efficiency of photosynthesis in low light condition

The steady maximum quantum yield and effective quantum yield from 0-86 d did not differ between treatments and control, which suggests that there was no photodamage to the zooxanthellae. When the MQY and EQY values are closer to one, this means the PSII of the

zooxanthellae is working properly and precisely (high efficiency). A measurement closer to zero means the photosystems are most likely stressed and/or damaged (low efficiency) (Warner et al., 1996). MQY and EQY essentially reveal the maximum potential of light capture occurring within the PS at any time. All measurements of MQY and EQY for this experiment were closer to one than zero, which suggests that the photosystems are working at relatively high efficiency. It could also imply the zooxanthellae chlorophyll had to work at high efficiency because there were low levels of light. In addition, the photosynthetic efficiency of PSII was not significantly different between treatments over the entire experiment, which further confirms the high efficiency of photosynthesis in the zooxanthellae.

The negative values of Q_m mean the effective quantum yield (light-acclimated) was larger than the maximum quantum yield (dark-acclimated). This is contrary to Roth and Deheyn's (2013) experimental findings of Q_m in a coral hot/cold experiment, in which maximum quantum yield was larger than effective quantum yield for zooxanthellae throughout their entire study. The results from Roth and Deheyn's (2013) study could be explained by coral skeletons scattering light and making their photic environment brighter even in lower light conditions (Roth and Deheyn, 2013; Wangpraseurt et al., 2020), while sea anemones do not have this characteristic.

Conclusions

This study implies a photoenhancement property of *A. sola* GFPs adapted to help them withstand low light conditions. This was only found in the lowest light condition that *A. sola* was exposed to in this experiment. *A. sola* is able to withstand PAR values of 57.80 and 108.27 PPFD without adjusting green fluorescence intensity. At 23.45 PPFD (lowest light condition), there is

evidence that implies a photoenhancement effect occurred in response to light shading. This study showed that the endosymbiotic zooxanthellae chlorophyll was able to perform photosynthesis at high efficiency despite low levels of ambient light and their density appeared to remain constant. The photoenhancement by the GFPs may have allowed the endosymbiotic zooxanthellae to perform photosynthesis at the high efficiency observed and provided the zooxanthellae with favorable conditions for photosynthesis.

The photoenhancement property of *A. sola* GFPs could explain their ability to live in the intertidal and subtidal zones within coastal waters with lower solar irradiance. The low light areas within the intertidal and subtidal zones in which *A. sola* inhabits include under rocks and sand and on pier pilings (Harris, 1991). *A. sola* tends to experience lower solar irradiance in the subtidal zone; therefore, GFP photoenhancement is more critical in this environment and is one important factor that allows *A. sola* to live in the subtidal zone and concentrate light enough for their endosymbiotic zooxanthellae to photosynthesize. When *A. sola* is unable to feed or is not receiving enough nutrients, their endosymbiotic zooxanthellae provide the necessary carbon to keep the sea anemones alive (Muscatine, 1980), which is why the photoenhancement of GFPs is such an important mechanism that is critical for living in low light environments. This study shows the photoenhancement property of GFPs in *A. sola* is one adaptation that explains the geographical and local distribution of the sessile organism, *Anthopleura sola*.

Future directions

Further experiments should be conducted to assess antioxidant capacity of purified fluorescent proteins (FPs) using a Total Antioxidant Status Assay Kit. The FPs should be purified from the tentacle samples taken on 0 d, 82 d (end of Phase I), and 106 d (end of Phase

II). Conducting studies on the antioxidant capacity of GFPs could show if GFPs were produced as an antioxidant in response to stress caused by the low light conditions. *A. sola*, similarly to corals, could be producing FPs to prevent oxidative stress in their tissue, and therefore, acting as a reactive oxygen species quencher (Palmer et al., 2009). In addition, tentacle samples taken on the same days (0, 82, and 106 d) could be used to generate excitation-emission matrices to find the excitation and emission wavelengths of the FPs and chlorophyll of *A. sola* and zooxanthellae respectively.

Field measurements of effective quantum yield and maximum quantum yield of the endosymbiotic zooxanthellae of *A. sola* would probably show whether the present experimental findings are consistent with *in situ* measurements.

In the future, it would be interesting to expose *A. sola* to even higher and lower PAR value treatments than used in the present study to better understand the effects of varying light levels on sea anemone green fluorescence as well as the photosynthetic efficiency of their endosymbiotic zooxanthellae. It would be beneficial to measure PAR in the intertidal and subtidal zones to investigate the amount of light available for the photosynthesis of the endosymbionts *in situ*. In addition, future studies should include more replicates of sea anemones to obtain stronger results.

A. sola experiences stressors simultaneously, not individually; therefore, a combination of stressors could reveal more changes in fluorescence intensity than seen in this experiment (Harris, 1991). Varying conditions of temperature and light, two components of solar radiation, and studying the effects on sea anemone fluorescence intensity and zooxanthellae photosynthesis efficiency would help test the possibility of GFPs functioning as a photoenhancement strategy. In addition, *A. elegantissima* could be a better test organism to use because of their binary fission

reproduction method creating genetically identical sea anemones rather than the genetically distinct *A. sola* (Francis, 1979). The inter-individual genetic variability of *A. sola* used in the present study could have contributed to variations; however, since *A. elegantissima* and *A. sola* are sibling-species, they may not differ in response to varying light intensities.

This study provides a foundation to expand upon the possible photoenhancement of GFPs in *A. sola* as an indicator of solar radiation stress to explain the geographical and local distributions of these sea anemones. It would be interesting to confirm and extend the present findings. In addition, further experiments might show that GFPs of *A. sola* could potentially be used as a proxy for solar radiation stress as a non-invasive field assay in subtidal and intertidal zones. GFPs of sea anemones could also be used as a proxy for other types of environmental and chemical stress, which would be interesting to further explore.

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